

The Independent Contribution of Bone and Erythrocyte Lead to Urinary Lead among Middle-aged and Elderly Men: The Normative Aging Study

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Plasma is the component of blood from which lead is free to cross cell membranes and cause organ toxicity. Plasma lead levels, however, are extremely low and difficult to measure. Urinary lead originates from plasma lead that has been filtered at the glomerular level; thus, urinary lead adjusted for glomerular filtration rate serves as a proxy for plasma lead levels. In this investigation we examined the interrelationships of lead levels in whole blood corrected by hematocrit [i.e., erythrocyte lead (EPb)], trabecular bone (TBoPb), cortical bone (CBoPb), and urine excreted over 24 hr (UPb); all samples were obtained from 71 middle-aged and elderly men with no known occupational lead exposures. Lead was measured by graphite furnace atomic absorption spectroscopy (blood), K-X-ray fluorescence (bone), and inductively coupled plasma mass spectroscopy (urine). Lead levels were generally low, with mean EPb, TBoPb, and CBoPb values of 13.8, 31.1, and 21.7 µg/g, respectively, and a median UPb value of 6.15 µg/day. In generalized additive models adjusted for body weight and creatinine clearance rate, both EPb and bone lead variables remained independently and significantly associated with UPb. This finding suggests that bone influences plasma lead in a manner that is independent of the influence of erythrocytic lead on plasma lead. Thus, the superiority of bone lead over blood lead in predicting some chronic forms of toxicity may be mediated through bone's influence on plasma lead. In addition, this study suggests that measurement of urinary lead might be useful as a proxy for plasma lead levels in studies of lead toxicity. **Key words:** blood, bone, environmental exposure, K-X-ray fluorescence, lead, urine. *Environ Health Perspect* 107:391–396 (1999). [Online 6 April 1999] <http://ehpnet1.niehs.nih.gov/docs/1999/107p391-396tsaih/abstract.html>

Although blood levels of lead have traditionally been used as a marker of lead dose in studies on the adverse effects of lead exposure, more than 99% of lead in blood is bound to erythrocytes (1–4). Plasma lead, the most toxicologically active component, is difficult to measure accurately because levels are extremely low and may be affected by contamination from hemolysis, sampling, and laboratory handling (3,5,6). The skeleton is the predominant endogenous storage site for lead; it contains more than 95% of the total amount stored (7–9). Lead stored in trabecular and cortical bone has a biological half life of >1 year and >5–10 years, respectively (10–12); thus, bone lead (especially lead in cortical bone) has been considered a proxy for cumulative exposure to lead (13–17). There is a growing awareness that bone lead is also the major endogenous source of lead (9,12,14,17–27). The release of lead from bone into the blood circulation is probably heightened during increased bone turnover, such as that associated with the rapid growth of childhood (28,29), pregnancy and lactation (30–34), menopausal osteoporosis (35,36), hyperthyroidism (37), and pathological states (38). Therefore, blood lead levels not only serve as an indicator of recent environmental

exposure, but also may reflect exposure from internal sources, primarily bone lead.

Despite the recent decline in environmental and occupational lead exposure in most developed countries (mainly due to the removal of lead from gasoline and the control of lead exposure in the workplace), general exposure to low doses of lead and pockets of high lead exposure are still common in the United States (39). With the development of *in vivo* K-X-ray fluorescence (KXRF) instruments, measurement of bone lead levels as an indicator of cumulative lead exposure in epidemiologic studies is now possible. Bone lead was found to be a better biomarker of lead dose than blood lead in recent studies of the relationship of low-level lead exposure to hypertension (40,41) and low birth weight (42). This finding raises concerns that blood lead levels not only inadequately represent levels of lead accumulated in bone but also inadequately represent levels of lead in plasma (the primary source of bioavailable lead to most body organs) and concerns that bone lead stores may independently influence plasma lead levels. Some kinetic models as well as empirical observations (43,44) have suggested the possibility of an

independent contribution of bone lead to plasma lead. More information is needed about the extent to which lead is mobilized out of bone in environmentally exposed individuals.

Although plasma lead provides critical information on the understanding of lead mobilization from bone, it is difficult to measure accurately. Urinary lead is an alternative to plasma lead. Urinary lead originates from plasma lead that has been filtered at the glomerular level; thus, urinary levels of lead that are adjusted for glomerular filtration rate serve as a proxy for plasma lead levels.

In this study, we test the hypothesis that bone and erythrocyte lead make independent contributions to urine lead by examining the interrelationships of lead levels in whole blood corrected by hematocrit [i.e., erythrocyte lead (EPb)], patella (TBoPb), tibia (CBoPb), and urine excreted over 24 hr (UPb) in a group of middle-aged and elderly men with no known occupational lead exposure. All research was approved by the Human Research committees of Brigham and Women's Hospital (Boston, MA) and the Department of Veterans Affairs Outpatient Clinic (Boston, MA).

Methods

Study population. Study participants were from the Normative Aging Study (NAS), a

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The authors gratefully acknowledge the research management of Soma Datta, Gail Fleischaker, Randi Heldman, Sybil Harcourt, and Nicola Lupoli.

Support for this research was provided by NIEHS R01 ES05257, NIEHS P42-ES05947, NIH NCRR GCRC M01 RR02635, NIEHS Center grant 2 P30 ES00002, and the Cooperative Studies Program/Epidemiology Research Information Centers of the Department of Veterans Affairs. Support was also provided by NIEHS 5 P42 ES-05947, with funding provided by the EPA. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH, or EPA. The KXRF instrument used in this work was developed by ABIOMED, Inc., of Danvers, MA, with support from NIH SBIR 2R44 ES03918-02.

Received 7 October 1998; accepted 25 January 1999.

longitudinal study of aging established by the Veterans Administration in 1961 (45). The study cohort initially consisted of 2,280 men from the Greater Boston, Massachusetts, area. The men were 21–80 years of age upon enrollment. All participants in the study were free of known chronic medical conditions at the time of enrollment; participants were excluded if they had any history of cancer, recurrent asthma, sinusitis, bronchitis, diabetes, gout, or peptic ulcer. Those with either a systolic blood pressure of >140 mmHg or a diastolic blood pressure of >90 mmHg were also disqualified. Since their enrollment in 1961–1968, participants have been reevaluated at 3- to 5-year intervals by a detailed core examination including collection of medical history information, routine physical examinations, laboratory tests, and questionnaires.

Since February 1987, each NAS participant has contributed a 24-hr urine specimen at his regularly scheduled examination. In addition, a blood sample used only for lead analysis has been collected at each visit since July 1988. Beginning in August 1991, NAS participants were recruited for a sub-study of KXRF bone lead measurements. Written informed consent was obtained from all participants.

In the present study, we randomly selected 100 men for lead analysis of 24-hr urine specimens, using a stratified sampling procedure based on quartiles of patella lead distribution among those NAS subjects who participated in the KXRF bone lead sub-study and provided 24-hr urine specimens.

Blood lead. Blood samples were obtained and analyzed for lead by graphite furnace atomic absorption with Zeeman background correction (ESA Laboratories, Chelmsford, MA). Values below the minimum detectable limit of 1 µg/dl were coded “0.” The instrument was calibrated with National Institute of Standards and Technology Standard Reference Material (NIST SRM 955a; lead in blood) after every 20 samples. Ten percent of samples were run in duplicate; at least 10% of the analyses were controls and 10% were blanks. In tests on reference samples from the Centers for Disease Control and Prevention (Atlanta, GA), precision [coefficient of variation (CV)] ranged from 8% for concentrations from 10 to 30 µg/dl to 1% for higher concentrations. As compared to a NIST target of 5.7 µg/dl, 24 measurements by this method gave a mean ± standard deviation (SD) of 5.3 ± 1.23 µg/dl.

Bone lead. Bone lead was measured in each subject's midtibia shaft and patella with a KXRF instrument (ABIOMED, Inc., Danvers, MA). The tibia and patella have been targeted for bone lead research

because these two sites are considered representative of the two main bone compartments—cortical and trabecular bone, respectively. A technical description and the validity specifications of this instrument have been published elsewhere (46,47). Radiation doses are minimal, with effective doses being a fraction of those associated with standard radiographs (48). This KXRF instrument uses ^{109}Cd to provoke the emission of fluorescent photons from target tissue that are then detected, counted, and arrayed on a spectrum (46). The net lead signal is determined after subtraction of Compton background counts by a linear least-squares algorithm. The lead fluorescence signal is then normalized to the elastic or coherently scattered γ -ray signal, which arises predominantly from the calcium and phosphorus present in bone mineral. The unit of measurement so derived is micrograms of lead per gram of bone mineral (micrograms per gram).

Since the inception of this study in 1991, KXRF instrument design has improved with the adoption of a 30-mCi ^{109}Cd spot-source design and 180° backscatter geometry (49,50). Improvements have also been made in the calibration of the original KXRF instrument used for this study. Furthermore, the original instrument is only slightly less precise than the newer instruments. For example, 10 repeated measurements of an 11.6-µg/g calibration phantom in water (which stimulates the *in vivo* situation) by our original KXRF instrument and a 30-mCi ^{109}Cd spot-source instrument gave mean ± SD results of 9.2 ± 3.7 and 10.0 ± 3.3 µg/g, respectively (50). Thus, we continued to use our original instrument to preserve the comparability of all measurements. Every week, a 10-µg/g phantom [true concentration verified by inductively coupled plasma mass spectrometry; see Aro et al. (50)] was positioned and measured 10 consecutive times overnight as a first-order calibration check; analysis of means and SDs did not disclose any significant shift in accuracy or precision during the life of this study.

Because the instrument provides a continuous unbiased point estimate that oscillates around the true bone lead value, negative point estimates are sometimes produced when the true bone lead value is close to 0. The instrument also provides an estimate of the uncertainty associated with each measurement that is obtained by a counting statistic modified by the goodness-of-fit for the curve function used to strip lead fluorescent peak counts from the underlying background. It is equivalent to a single SD if multiple measurements are taken. Although a minimum detectable

limit calculation of twice this value has been proposed for interpreting an individual's bone lead estimate (49), retention of all point estimates makes better use of the data in epidemiological studies (51). The technicians measuring bone lead were blinded to the participants' health status.

Urinary lead. One week before his scheduled medical examination, each participant received a 4-liter container by mail for 24-hr urine collection at home. Five representative containers from each batch of 100 urinary containers were prechecked for lead concentration (no lead was found). Each container was accompanied by instructions and a questionnaire about the collection of the sample, including time of collection, missed collections, spillage, and subject's use of medication. Collection began after the first void of the morning and continued through the first void of the subsequent morning. Urine samples were collected in the presence of an antioxidant (3 ml sodium metabisulfite) and an acid (15 ml, 6 M hydrochloric acid) to achieve a pH between 2 and 3. The concentration of lead in sodium metabisulfite and in HCl has been measured by our laboratory; values of 0.076 and 0.077 ng/ml, respectively, were found. The total amount of lead contamination from HCl and metabisulfite was 1.37 ng. Contamination was insignificant based on the lowest amount of lead measured in urine (0.73 µg). The samples were stored at -20°C before assay, then thawed, aliquotted, digested with HNO_3 at room temperature, and analyzed by inductively coupled plasma mass spectrometry (Sciex Elan 5000, Perkin-Elmer, Norwalk, CT) with standard instrument-operating and data-collecting parameters using the isotope dilution procedure (52). Prior to analysis, the mass spectrometer setting and nebulizer flow rate were optimized to give maximum peak intensity for lead. A solution (10 ng/ml) of enriched isotope spike (^{206}Pb ; NIST SRM 983) was mixed with the sample online by means of a mixing Tee and a mixing coil. Data were expressed as the average of five replicate measurements. The accuracy of the isotope dilution procedure has been verified using urine samples from interlaboratory comparison study samples from the Quebec Interlaboratory Comparison Program (Le Center de Toxicologie du Quebec), whose target values were determined by electrothermal atomic absorption spectroscopy. The percentage error calculated for Pb determination in urine samples ranged from -4.26 to 2.6%. Quality control and quality assurance procedures included analyses of procedural blanks to monitor contamination and NIST SRM 2670e (toxic elements in urine)

to monitor the accuracy and recovery rates for each analytic batch. The concentration in the procedural blanks was 0.03 ng/ml with a detection limit of 0.02 ng/ml. Recovery of these quality control standards is between 95 and 105%, and the precision (relative standard deviation) of the Pb determination is approximately 1%.

Creatinine. We used the Beckman Creatinine Analyzer 2 (Beckman, Brea, CA) to measure creatinine in serum and in archived (frozen) 24-hr urine samples by routine laboratory method (Jaffe method). This method of analysis has a detection limit of 0.2 mg/dl with intra-assay CV of 2.4% at 17.7 mg/dl and inter-assay CV of 3.1% at 17.6 mg/dl.

Statistical analysis. Any 24-hr urine sample with a volume of <750 ml or with a collection time of <20 hr was excluded from this analysis to ensure quality. We estimated glomerular flow rate from the clearance rate of endogenously produced creatinine. Creatinine clearance rate (CCr) was calculated by the following formula: CCr (ml/min) = [total amount of urinary creatinine over 24 hr (mg)]/[serum creatinine concentration (mg/ml) × collection time (min)]. Lead in blood is composed of two parts—bound to red blood cell (erythrocyte) and free in plasma. Because more than 99% of lead in whole blood is bound to erythrocytes (3,4), the part of plasma lead is negligible and whole blood lead (BPb) concentration can be inferred as the approximate of erythrocyte lead (EPb) concentration weighted by the percentage of blood volume occupied by erythrocytes, i.e., BPb ≈ EPb × hematocrit. Therefore, EPb concentration was calculated by the following formula: EPb (μg/dl) = [whole blood lead concentration (μg/dl)]/[hematocrit (%)].

The relationship between blood lead and plasma lead is nonlinear at blood lead levels of >40 μg/dl in adults (3,4). The relation

between blood lead and urinary lead among lead workers is also nonlinear, according to a recent study (43). Little has been reported on those relationships in nonoccupationally exposed individuals. A recent study on environmentally exposed individuals reported a poor linear relationship between blood lead and urine lead, particularly at lower range of exposures (53). It was considered possible that the relation between blood lead and urinary lead would be nonlinear in the population we studied. Before we examined the independent contribution of bone lead to urinary lead, we needed to control for possible nonlinearities in their relationship. One analytical method well suited to such a task is the generalized additive model (GAM) (54).

A standard linear regression model assumes that $E(Y) = \beta_0 + \beta_1 X_1 + \dots + \beta_p X_p$, where $E(Y)$ denotes the expected value of Y and the predictor variables (X_1 – X_p) may be transforms of underlying variables or products of those variables. Although this approach can incorporate nonlinear forms of the dependencies, it is necessary to specify the functional form of those dependencies. If we believe we know the correct covariates but not necessarily the correct functional forms, the GAM offers a nonparametric regression alternative. It assumes that $E(Y) = \beta_0 + S_1(X_1) + \dots + S_p(X_p)$.

In this case, $S_1(X_1)$ is a smooth function of X_1 that is fit nonparametrically. A nonparametric smoother is a tool for summarizing the trend of a response measurement Y as a function of one or more predictor measurements. Most smoothers are generalizations of weighted moving averages. These predict the expected value of Y at X_k as the weighted mean of the values of the Y_i corresponding to all the X_i in a symmetric neighborhood around X_k . The weights decline with distance from the center of the neighborhood. The properties of moving average smoothers have been extensively discussed (55,56) and the utility of GAMs for environmental epidemiologic studies has been well demonstrated (57–59). Linear terms are a special subset of smoothers, and linear variables can be mixed with nonparametric smoothers in this model. This approach not

only offers descriptive advantages, but also is of value as an adjustment tool in the determination of an effect of interest. As a visual summary, the smoother is helpful and may suggest possible parametric forms.

The loess smoothed parameter (60) was used in this analysis with selection of a parameter that minimizes Aikake's information criterion (61). Twenty-four-hour urinary lead was regressed on smooth functions of age, EPb, CCr, and body weight. We chose the best smoothing parameter for each variable in models that did not contain bone lead variables. Patella lead and tibia lead were then examined to see whether they made any independent contribution to 24-hr urinary lead levels. Only subjects with complete data sets were included in the analysis, and all regressions were estimated in S-plus with the GAM function.

Results

Two subjects with missing data on EPb and urinary creatinine were eliminated from the analysis, as were four subjects with 24-hr urine volume of <750 ml or with <20 hr of urine collecting time. In addition, 17 subjects with reduced renal function (a serum creatinine concentration of ≥1.5 mg/dl or a CCr of <50 ml/min) were excluded. Generalized ESD many-outlier procedures (62) were run on the remaining 77 subjects. Six subjects, who had an EPb of >47 μg/dl, a TBoPb of ≥90 μg/dl, or a body weight of >130 kg, were identified as outliers. After these outliers were excluded, 71 subjects remained for regression analysis. The means, SDs, and ranges for the variables of interest are listed in Table 1, and Spearman correlations are summarized in Table 2. TBoPb is significantly higher than CBoPb. EPb had the strongest correlation with UPb, followed by CCr, TBoPb, and CBoPb. TBoPb was a stronger correlate of EPb than was CBoPb. UPb was positively correlated with CCr, probably because the amount of lead excreted into urine is modified by renal function. Body weight was negatively correlated with TBoPb and CBoPb and was positively correlated with CCr and UPb.

Table 1. Lead levels in whole blood, erythrocytes, bone, and 24-hr urine from 71 middle-aged and elderly men in the Normative Aging Study

Variable	Mean ± SD	Range
Age (years)	67.77 ± 6.1	53.01–81.67
Body weight (kg)	85.42 ± 13.4	59.47–128.03
Creatinine clearance rate (ml/min)	91.73 ± 25.7	55.18–162.92
Whole blood lead (μg/dl)	5.94 ± 3.0	0.00–15.00
Erythrocyte lead (μg/dl) ^a	13.81 ± 7.0	0.00–35.90
Patella lead (μg/g)	31.1 ± 15.1	6.0–74.0
Tibia lead (μg/g)	21.7 ± 10.9	5.0–49.0
Urinary lead (μg/day)	5.69 (1.9) ^b	0.73–19.57

SD, standard deviation.

^aErythrocyte lead calculated by dividing whole blood lead by hematocrit values.

^bGeometric mean.

Table 2. Spearman correlations between biological markers of lead dose, age, and creatinine clearance in the 71 middle-aged and elderly men in the Normative Aging Study

Variable	Age	Erythrocyte lead	Patella lead	Tibia lead	Creatinine clearance rate	Body weight
Urinary lead	0.077	0.673**	0.347**	0.278**	0.514**	0.053
Age		0.071	0.126	0.208	-0.152	-0.176
Erythrocyte lead			0.362**	0.251*	0.217	0.136
Patella lead				0.694**	-0.037	-0.287
Tibia lead					0.038	-0.279*
Creatinine clearance rate						0.250*

* $p < 0.05$; ** $p < 0.01$.

Neither a linear term nor a smooth function of age was a significant predictor of UPb. Moreover, the model fit was the same with or without age. Age was therefore dropped from these models. A GAM plot showed a somewhat nonlinear EPb–UPb relationship after adjustment for CCr and body weight (Fig. 1). After adjustment for EPb, CCr, and body weight, TBoPb and CBoPb were significant predictors of UPb in separate models. According to Akaike's information criterion (61), CBoPb fit as well as TBoPb. GAM plots from both models indicated that lead stored in these two bone sites had different relations with UPb. The TBoPb–UPb relation was linear ($\beta = 0.05$) (Fig. 2) and the mean contribution of lead from trabecular bone to urine was 1.6 $\mu\text{g/day}$. In contrast, the CBoPb–UPb relation was linear only above a threshold of 24 $\mu\text{g/g}$, and no CBoPb–UPb association was evident at CBoPb values below 24 $\mu\text{g/g}$. Only 38% of the subjects had CBoPb >24 $\mu\text{g/g}$. On the basis of this finding, we took a different approach and refit the tibia lead model. Because of the observed threshold

effect of tibia lead on UPb, a new variable was introduced to replace the loess function of tibia lead. This new variable, *hightib*, has a value of 0 if CBoPb is <24 and has a value of CBoPb–24 otherwise. The results are listed in Table 3. The model with a linear term of *hightib* ($\beta = 0.12$) fit as well as the patella lead model. Mean contribution of lead from cortical bone to urine was 0.43 $\mu\text{g/day}$. The nonlinear EPb–UPb relation remained significant in both the patella lead and new tibia lead models.

Discussion

The results of the present study indicate a strong nonlinear relation of EPb to UPb as well as an independent association of skeletal lead to UPb. After adjustment for EPb, CCr, and body weight, TBoPb and CBoPb were significant predictors of UPb in separate models.

The middle-aged and elderly male participants in this study had no known occupational exposure to lead, and their environmental exposures to lead were presumably through contaminated drinking

water, air, and food. A simple kinetic model of lead in the body can be described as equilibrium between bone and blood. However, in this study population, skeletal stores may be the source of increased levels of lead circulating in plasma and therefore of increased lead excretion in urine because of increased bone mineral loss resulting from aging (Fig. 3). The significant independent relations of UPb with bone lead, which we observed in the GAM, is consistent with the potential role of the skeleton as an important endogenous source of labile lead that may not be adequately discerned by examining whole blood lead or EPb. The direct evidence of contribution of lead from skeleton to urine and to blood has been shown by lead tracer methods (12,63) and stable isotope methods (25,64,65), respectively. The stable lead isotope method has the ability to distinguish between lead from endogenous sources (i.e., skeleton) and from dietary sources. Forty-one to 73% of blood lead originated from bone in a group of adult women (25), as opposed to 20% of total blood attributable to bone lead in a nonhuman primate (64). A recent study of nonhuman primates using the same technique observed a significant increase in bone-derived lead in maternal blood in late pregnancy (65).

Bone lead turnover is determined by lead exchange at bone surfaces and factors affecting bone remodeling (66,67). Trabecular bone has a larger surface area and a greater volume of blood delivered per unit of time than cortical bone. In addition, there are more active osteons per gram in trabecular bone to carry out resorption and deposition than in cortical bone. Consequently, endogenous exposure would be expected to relate more closely to trabecular than to cortical lead, and trabecular bone would be expected to relate more closely to circulating lead and urinary lead. Our findings that TBoPb was a stronger correlate of EPb and UPb than was CBoPb (Table 2) and contributed more lead in urine (1.6 $\mu\text{g/day}$ from TBoPb and 0.43 $\mu\text{g/day}$ from CBoPb) are consistent with these ideas. Similar findings regarding the blood lead–trabecular bone lead association

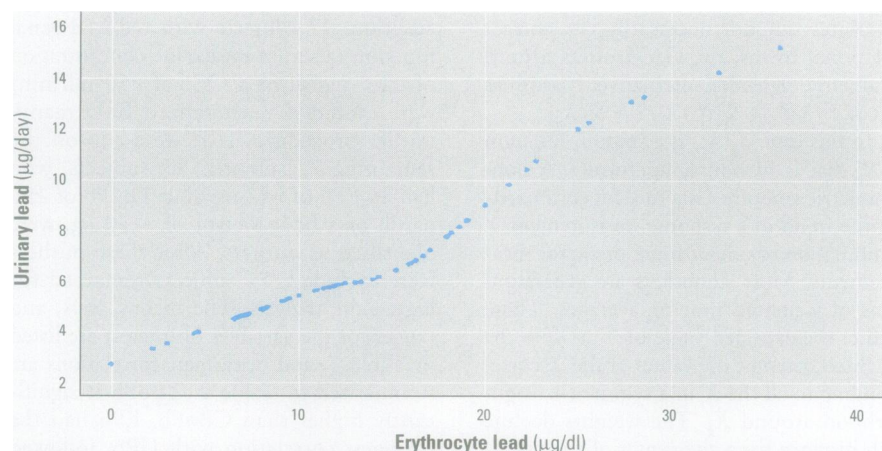


Figure 1. The smoothed relationship of erythrocyte lead to urinary lead after adjustment for smoothed terms of creatinine clearance and body weight.

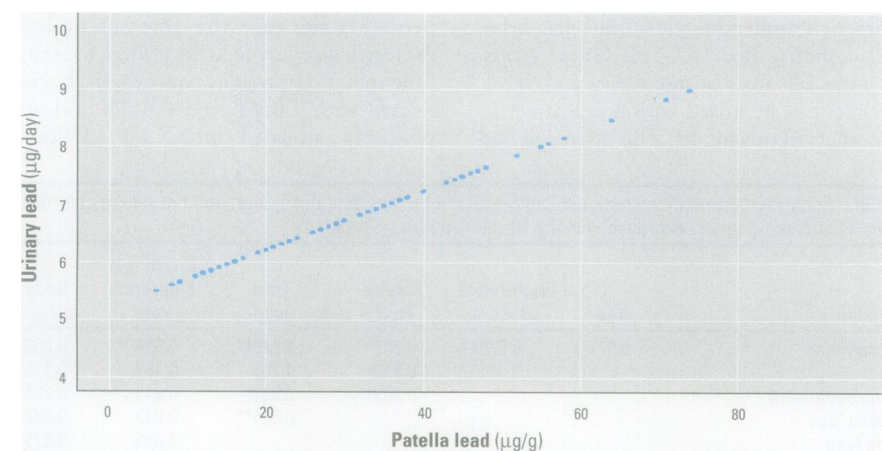


Figure 2. The relationship of patella lead to urinary lead after adjustment for smoothed terms of erythrocyte lead, creatinine clearance, and body weight.

Table 3. Generalized additive models relating bone lead measures to urine lead^a

	Coefficient	SE
Tibia lead model		
Tibia lead	0.12*	0.05
Patella lead model		
Patella lead	0.05*	0.02

SE, standard error.

^aEach model adjusts for smooth functions of hematocrit-adjusted blood lead, body weight, and creatinine clearance.

* $p < 0.05$.

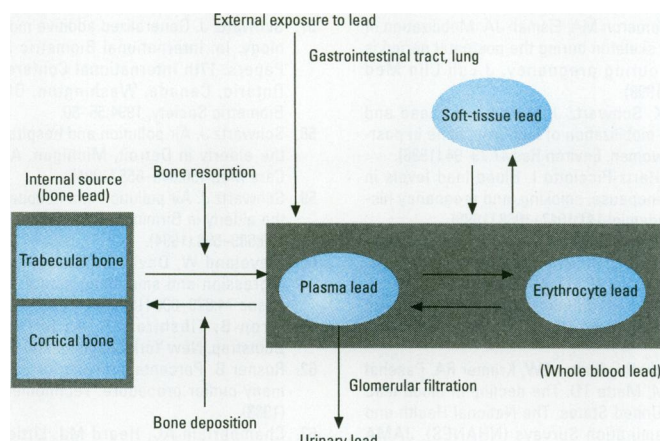


Figure 3. Conceptual model of plasma lead exchange in the human body.

have been reported in a previous cross-sectional study in the NAS population (68) and in a case-control study of postpartum women in Mexico City (69). In addition, a recent study reported that bone lead (particularly trabecular bone lead) exerts an additional independent influence on plasma lead after controlling for whole blood lead in individuals with no history of occupational lead exposure (70). Together, these findings support the argument that lead in trabecular bone is more available for mobilization and has more influence on the amount of lead excreted in urine than in cortical bone.

As depicted in Figure 3, lead in blood is divided between erythrocyte and plasma compartments. Although the lead-binding capacity of erythrocytes is usually assumed to have specific limits (71), factors governing the relative partitioning between lead in plasma and erythrocyte are not well known. Marcus (71) points out the existence of other possible models in addition to the saturation model. Leggett (29) also notes that the saturation concentration may depend on the levels and duration of exposure. Bone lead is an important endogenous source of plasma lead, whereas whole blood lead is an important determinant of plasma lead (Fig. 3). Cake et al. (72) noted that among active workers in a lead recycling facility, the serum/blood lead ratio correlated significantly and positively with bone lead but not with whole blood lead. They hypothesized that part of the variation in serum/blood lead ratio could be attributed to lead released from bone and therefore high bone lead levels produce high serum/blood lead ratios. Berghdahl and Skerfving's (73) recent observation among retired lead smelter workers undermined the hypothesis proposed by Cake et al. (72). However, the finding of a significant correlation of plasma/blood ratio to blood and bone lead in environmentally exposed adults (70) is in

good agreement with the Cake et al. (72) hypothesis. Discrepancies between these studies might be explained by the use of different target sites for bone lead measurement and different lead exposure levels.

In contrast, there are few reports on the relationships of BPb or PPb to UPb. Most of the observations were collected on occupationally exposed individuals. The relationship between BPb and UPb among lead workers was nonlinear in a recent study (43). Hirata et al. (44) noted that the correlation between the concentrations of PPb and of UPb exceeded that between the concentrations of BPb and UPb among lead workers with low levels of lead exposure. A physiologically based kinetic model predicted that the rate of lead excretion into urine is directly proportional to plasma concentration (66). Only one observation of the BPb-UPb relationship among environmentally exposed individuals is available (53). The authors noted a poor linear relationship between BPb and UPb, particularly at lower range of exposures (53). Although we were unable to directly investigate the relationship between UPb and PPb, the curvilinear relation (Fig. 1) and strong correlation between UPb and EPb in the present study are compatible with the findings of a nonlinear relationship as seen in previous studies.

The main source of external exposure to lead includes diet and air for individuals without occupational exposure. Tap water in a city like Boston, with old houses containing lead plumbing, is a significant dietary source of potential lead exposure. The mean whole blood lead levels in the NAS population is representative of the general population in the United States (74). A recent study in a subset of the NAS population found that ingestion of lead-contaminated tap water is an important predictor of elevated bone lead levels later in life (75). Lead in diet, in addition to bone lead and blood

lead, may contribute substantially to lead in urine. Our study was limited by the fact that dietary intake of lead was not measured and we were unable to distinguish between lead from the skeleton and lead from dietary sources. Nevertheless, in an aging population like the NAS, where endogenous exposure is the dominant contributor to whole blood lead, the influence of diet should be minimal.

Glomerular function, in addition to bone lead and EPb, is another critical determinant of the amount of lead excreted. Because it is possible that renal impairment influences lead levels in blood and urine, we excluded all subjects with significant renal impairment (serum creatinine levels of ≥ 1.5 mg/dl or a CCr of < 50 ml/min) and we further controlled for renal function by including the best smooth function of CCr in our models. Accordingly, it is unlikely that our results were significantly influenced by renal impairment.

In summary, our findings of independent contributions of bone and erythrocyte lead to urine lead indicate that the skeleton is an important endogenous source of lead exposure in environmentally exposed subjects. Moreover, UPb originating from PPb may be an alternative measure of the most metabolically relevant fraction of lead in blood that will be useful in assessing the potential hazard in populations where endogenous exposure is the dominant contributor to whole blood lead. Further research is needed to examine the use of UPb as an alternative to PPb in examining the mechanism of bone lead toxicity in environmentally exposed subjects.

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